

## CASE REPORT

### Determination of propofol by GC/MS and Fast GC/MS-TOF in two cases of poisoning

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20    **Abstract**

21           Two cases of suspected acute and lethal intoxication caused by propofol were delivered by  
22   the judicial authority to the Department of Sciences for Health Promotion and Mother-Child Care in  
23   Palermo, Sicily. In the first case a female nurse was found in a hotel room, where she lived with her  
24   mother; four 10 mg/mL vials and two 20 mg/mL vials of propofol were found near the decedent  
25   along with syringes and needles. In the second case a male nurse was found in the operating room  
26   of a hospital, along with a used syringe. In both cases a preliminary systematic and toxicological  
27   analysis (STA) indicated the presence of propofol in the blood and urine. As a result, a method for  
28   the quantitative determination of propofol in biological fluids was optimized and validated using a  
29   liquid-liquid extraction protocol followed by GC/MS and Fast GC/MS-TOF. In the first case, the  
30   concentration of propofol in blood was determined to be 8.1 µg/mL while the concentration of  
31   propofol in the second case was calculated at 1.2 µg/mL. Additionally, the tissue distribution of  
32   propofol was determined for both cases. Data emerging from the autopsy findings, histopathological  
33   exams as well as the toxicological results aided in establishing that the deaths were due to  
34   poisoning, however the manner of death in each were different: homicide in Case 1 and suicide in  
35   Case 2.

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41   **Keywords** Propofol · Poisoning · Systematic and toxicological analysis · Tissue distribution · Cause  
42   of death · GC/MS-TOF

## 43    **Introduction**

44            Propofol (2,6-diisopropylphenol), a sedative-hypnotic agent used for the induction of  
45    anesthesia and for sedating mechanically ventilated patients in intensive care units [1,2], is now  
46    increasingly being used for conscious sedation during endoscopic procedures. Propofol is an  
47    extremely rapid-acting intravenous anesthetic. Its advantages include less residual postoperative  
48    sedation and less psychomotor impairment compared to the barbiturates and less incidence of  
49    nausea and vomiting [3]. The blood concentration required for induction of anesthesia is generally  
50    2-10 µg/L, while a concentration of 2-4 µg/L is sufficient to maintain it [4,5]. Propofol produces  
51    dose-dependent cardiovascular and respiratory depression with a profile similar to methohexital.  
52    Side effects include pain on injection, involuntary muscle movements, coughing, and hiccoughing  
53    [6]. It has been associated with fatal heart failure both in children [7] and in adult patients with head  
54    injuries [8]. In fact, the constellation of myocardial failure, metabolic acidosis, and rhabdomyolysis  
55    in children receiving propofol infusions for more than 48 hours has been termed the *propofol*  
56    *infusion syndrome* [9,10]. Propofol is known to induce hypertriglyceridemia, severe enough to  
57    cause pancreatitis, but only when used at a rate exceeding 100 µg kg<sup>-1</sup>min<sup>-1</sup> for prolonged periods  
58    [11]. Propofol is also associated with abuse and dependency, especially among health care  
59    professionals [12-14], because of its rapid narcotic effect causing euphoria and sexual hallucinations  
60    [15].

61            Several fatal cases of poisoning have been reported [13-20]; in these cases a high variability  
62    in the blood concentration of propofol has been observed (from 0.08 to 8.7 µg/L) [4].

63            Two cases of suspected lethal intoxication caused by propofol were delivered by the judicial  
64    authority to the Department of Sciences for Health Promotion and Mother-Child Care in Palermo,  
65    Sicily in 2014. A GC/MS method previously developed and validated in our laboratory [21] was  
66    applied for the determination of volatile organic compounds (VOC) and the systematic

67 toxicological analysis (STA) on blood and urine collected from the two cases. In both cases STA  
68 indicated the presence of propofol in blood and urine. A method was therefore optimized and  
69 validated for the quantitative determination of propofol in the biological fluids using a liquid-liquid  
70 extraction protocol followed by GC/MS and Fast GC/MS-TOF. Blood, urine, bile and tissue  
71 concentrations were determined for both cases [22].

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### 73 **Case history**

74 First case: female, nurse, 41 years old, sitting on a chair near a bed in a hotel room. Four 10  
75 mg/mL vials and two 20 mg/mL vials of propofol were found near the decedent together with  
76 syringes and needles. Signs of acupuncture on the left elbow, forearm, hand and foot were noted.  
77 Blood, urine, bile, brain and liver were obtained at the autopsy.

78 Second case: male, nurse, 55 years old, found lying in an operating room with a syringe  
79 nearby. Sign of acupuncture on the right ankle. Blood, urine, brain, liver and kidney were obtained  
80 at the autopsy.

81

### 82 **Materials and methods**

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#### 84 *Reagents, chemicals and standards*

85 All reagents were of analytical grade and were stored as indicated by the supplier. Ethyl  
86 acetate, 2-propanol, dichloromethane, methanol, ammonia, hydrochloric acid 37%, sodium chloride,  
87 sodium bicarbonate, sodium carbonate, anhydrous sodium sulfate sodium hydroxide, O,N-  
88 bis(trimethylsilyl)trifluoroacetoamide-trimethylchlorosilane (BSTFA-1% TMCS), pH 6 buffer were  
89 purchased from Sigma-Aldrich (St. Louis, MO, USA); Thymol and sodium sulfate were obtained  
90 from Farmalabor (Canosa di Puglia, Italy). MethElute Reagent 0.2 M in methanol (TMAH) was  
91 from Thermo Scientific (Waltham, MA, USA). Propofol was purchased from Archimica S.p.a

92 (Origgio, Italy). Water ( $18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$ ) was prepared by a Milli-Q System (Millipore, Darmstadt,  
93 Germany); other common chemicals were of the highest purity commercially available.

94 Stock solutions of propofol (0.1, 0.25, 0.50, 1, 2, 3, 10, 20, 25, 50, 100  $\mu\text{g/mL}$ ) and thymol  
95 (IS; 10, 100, 1000  $\mu\text{g/mL}$ ) were prepared in methanol and stored at 4 °C.

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97 *Systematic and toxicological analysis (STA) [21]*

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99 *Blood, urine and bile sample preparation*

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101 Blood (1 mL), urine (1 mL) or bile (250  $\mu\text{L}$ ) was added with IS (100  $\mu\text{L}$ , 10  $\mu\text{g/mL}$ ),  
102 saline solution (up to 2 mL), bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) and  
103 extracted with ethyl acetate (4 mL). The mixture was put on a rotary shaker (20 min, 15 rpm)  
104 and then centrifuged (5 min, 5000 rpm). The organic phase was separated, sodium sulfate was  
105 added and after centrifugation (5 min, 5000 rpm) the supernatant was withdrawn and the  
106 solvent evaporated. The residue was dissolved in ethyl acetate (100  $\mu\text{L}$ ) before the analysis.

107 To evaluate specificity blood, urine or bile working standard solutions were prepared as  
108 follows: 100  $\mu\text{L}$  of propofol standard solution (10  $\mu\text{g/mL}$ ) were placed in vial and the solvent  
109 evaporated. Blank blood (1 mL), blank urine (1 mL) or blank bile (250  $\mu\text{L}$ ), IS (100  $\mu\text{L}$ ,  
110 10 $\mu\text{g/mL}$ ), saline solution (up to 2 mL), bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9)  
111 were added and the mixtures extracted as described before.

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113 *Hydrolysis of propofol glucuronide and sulfate in urine and bile samples*

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115           The sample of urine (1 mL) or bile (250  $\mu$ L) was added with saline solution until a volume  
116 of 2 mL and 1 mL of 6N hydrochloric acid was added. The mixture was heated at 105 °C for 1 h.  
117 After cooling, IS (100  $\mu$ L, 10  $\mu$ g/mL) was added, pH was adjusted to 8 and bicarbonate-  
118 carbonate buffer (50 mg, 2/1 w/w, pH 9) was added. Then the mixtures were extracted as  
119 described before.

120           Hydrolyzed urine or bile working standard solutions were prepared as follows: 100  $\mu$ L  
121 of propofol standard solution (10  $\mu$ g/mL) were placed in vial and the solvent evaporated.  
122 Blank urine (1 mL) or blank bile (250  $\mu$ L) and saline solution until a volume of 2 mL were  
123 added; the mixture was heated at 105 °C for 1 h. After cooling, IS (100  $\mu$ L, 10  $\mu$ g/mL) was  
124 added, pH was adjusted to 8 and bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) was  
125 added. Then the mixtures were extracted as described before.

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#### 127 *Tissue sample preparation*

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129           Each sample was homogenized with a blender or ball mill, depending on the quantity of  
130 material. The deproteinization of the biological matrix was performed by means of an ultrasonic  
131 bath: 100 mg of tissue (brain, liver or kidney) previously added with 4 mL of saline solution,  
132 bicarbonate-carbonate buffer (50 mg, 2/1 w/w, pH 9) and 100  $\mu$ L of IS (10  $\mu$ g/mL) were  
133 sonicated for 15 minutes at room temperature. After 5 min centrifugation, a clear supernatant was  
134 separated and extracted with ethyl acetate (4 mL). The mixture was placed on a rotary shaker  
135 (20 min, 15 rpm) and then centrifuged (5 min, 5000 rpm). The organic phase was separated,  
136 anhydrous sodium sulfate was added and after centrifugation (5 min, 5000 rpm) the

137 supernatant was withdrawn and the solvent evaporated. The residue was dissolved in ethyl  
138 acetate (100  $\mu$ L) before the analysis.

139 Tissue working standard samples were prepared as follows: 100  $\mu$ L of propofol  
140 standard solution (10  $\mu$ g/mL) were placed in vial and the solvent evaporated. Blank tissue (100  
141 mg), IS (100  $\mu$ L, 10  $\mu$ g/mL), saline solution (4 mL), bicarbonate-carbonate buffer (50 mg, 2/1  
142 w/w, pH 9) were added and the mixtures extracted as described before.

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#### 144 *GC/MS*

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146 The analyses were performed on a HP6890 Series II GC system, with a split-splitless  
147 injection system and an MSD HP5973 MS detector (Agilent Technologies, Santa Clara, CA, USA)  
148 operated in electron ionization (EI) mode (70 eV). The GC was equipped with a Rxi®-5Sil MS (5%  
149 diphenyl/95% dimethyl polysiloxane, 30 m x 0.25 mm i.d., film thickness 0.25  $\mu$ m) capillary  
150 column (Restek, Bellefonte, PA, USA).

151 GC/MS conditions: splitless; solvent delay, 3.5 min; injector temperature, 280°C; interface  
152 transfer line, 280°C; ion source, 280°C; oven temperature program, initial 70°C, 40°C/min up to  
153 110°C, then 15°C/min up to 300°C (3 min). Helium was used as the carrier gas at a flow rate of 1.2  
154 mL/min. The MS detector was operated in the scan mode, acquiring ions from  $m/z$  50 to 550. The  
155 total analysis time was 21 min.

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#### 157 *GC/MS-TOF*

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159 The analyses were performed on a Dani Master GC system, with a split-splitless injection  
160 system and a Dani Master TOF Plus detector (Dani Instruments, Cologno Monzese, Italy) operated

161 in electron ionization (EI) mode (70 eV). The GC was equipped with a Rxi<sup>®</sup>-5ms (Crossbond<sup>®</sup>, 5%  
162 diphenyl/95% dimethyl polysiloxane, 10 m x 0.10 mm i.d., film thickness 0.15 µm) capillary  
163 column (Restek, Bellefonte, PA, USA).

164 The GC/MS conditions: split ratio 100:1; injector temperature, 250°C; interface transfer line,  
165 280°C; ion source, 200°C; oven temperature program, initial 70°C, 20°C/min up to 200°C, then  
166 30°C/min up to 300°C (17 s). Helium was used as the carrier gas at a flow rate of 0.5 mL/min. The  
167 MS detector was operated in the scan mode, acquiring ions from  $m/z$  50 to 550. The total analysis  
168 time was 8 min. The selected ions were 163 and 178 for propofol and 135 and 150 for the IS.

169

#### 170 *Method validation*

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172 The specificity, accuracy, precision and linearity as well as the limit of detection (LOD) and limit of  
173 quantitation (LOQ) were evaluated using blood as matrix.

174 The specificity was assessed by extracting control (blank) blood, urine, bile, brain, liver and kidney  
175 samples. The lack of interfering peaks at the same analyte retention times conferred acceptable selectivity.

176 The linearity of the response of the GC/MS-TOF analysis was assessed for propofol by plotting  
177 drug/IS peak area ratios *versus* the total amount of drug in the standard solutions, with intervals of 25–2000  
178 total ng of analyte (25, 50, 75, 150, 200, 500, 1250, 1500, 2000 ng<sub>tot</sub>). The calibration curve ( $y = 0,0007x -$   
179  $0,0204$ ) gave good correlation coefficients ( $R^2 > 0.9925$ ) over the whole range.

180 Accuracy was expressed as the per cent recovery (%REC) evaluated by analyzing, in triplicate, two  
181 standard propofol solutions (500 to 1250 ng<sub>tot</sub>). The averaged results were found to be satisfactory (mean  
182 %REC 86.6 at 500 and 111.1 at 1250 ng<sub>tot</sub>).

183 Two standard solutions (500 to 1000 ng<sub>tot</sub>) were analyzed five times in the same day and over 5 days  
184 in order to evaluate the precision of the method. The intraday and interday %CV were respectively 7.55 and  
185 9.82% at 500 ng<sub>tot</sub>; 8.51 and 5.03% at 1000 ng<sub>tot</sub>. The obtained data demonstrated adequate reproducibility.



186 The LOD and LOQ were also evaluated and were found to be 10 and 25 ng evaluated as the  
187 concentration of the analyte which gives a signal to noise ratio of at least 3 and 10 respectively.

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## 189 **Results and discussion**

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191 STA was carried out on the biological samples of the two cases received. Blood and urine of both  
192 cases were evaluated; however bile was available only in the first case. Case 1 did not test positive for VOC;  
193 however Case 2 had a blood alcohol concentration of 0.2 g/L. Other non-volatile substances identified in the  
194 cases are reported in Table 1. As noted caffeine, cotinine and nicotine were identified in both cases and are  
195 considered toxicologically irrelevant. Of interest is the presence of a chromatographic peak whose mass  
196 spectrum correlated to silanized propofol (Fig. 1). Based on the nature of the two cases, the laboratory  
197 proceeded with developing an analytical method for the quantification of propofol in biological  
198 fluids and tissues.

199 Due to the low recoveries obtained with the original SPE method [21], a liquid-liquid  
200 extraction protocol was developed with ethyl acetate at pH 9 (bicarbonate/carbonate buffer) to  
201 optimize the extraction of propofol in the organic phase. Thymol was chosen as internal standard.  
202 The extracts were silanized using O,N-bis(trimethylsilyl)trifluoroacetoamide-trimethylchlorosilane  
203 (BSTFA-1% TMCS) as in the STA analysis, but due to the low reproducibility of the results by  
204 GC/MS, the determination of propofol after the liquid-liquid extraction protocol without  
205 derivatization was carried out. Unfortunately, two interfering species were detected: capric acid in  
206 blood and nicotine in urine samples (Fig. 2).

207 At this point the chromatographic system was completely changed, using Fast GC/TOF,  
208 with narrower and shorter capillary columns. The fast heating and cooling rate of the GC oven and  
209 the fast acquisition rate of the MS detector, allow high sensitivity and resolution and the  
210 chromatographic separation results enhanced although the shortness of the column. In these  
211 conditions, the peak of propofol was completely separated from those of capric acid and nicotine

212 (Fig. 2). The method was validated using blood as matrix showing suitable selectivity, accuracy,  
213 precision, LOD, LOQ and linearity in the concentration ranges requested for propofol determination  
214 in biological specimen [5, 12-22].

215 The optimized method was applied for the determination of propofol in the biological  
216 specimens from the two cases. Urine and bile samples were hydrolyzed because it is known that  
217 most of propofol is conjugated with glucuronic acid [5]. A chromatogram obtained for the analysis  
218 of blood of Case 1 is depicted in Figure 3.

219 The results obtained analyzing the biological samples from the two cases are reported in  
220 Table 2.

221 The interpretation of the results should be made with particular caution. It is still widely  
222 debated whether propofol can be used to suicidal overdose. Several coroners believe that it is not  
223 possible to commit suicide with propofol because the maximum voluntarily injectable quantity of  
224 propofol before losing consciousness is not sufficient to cause death [23]. Death could be caused by  
225 a continuous intravenous infusion of the drug, with multiple organs failure mimicking propofol-  
226 related infusion syndrome. The two cases show very different propofol concentrations especially in  
227 blood and urine. In Case 2 propofol levels, found in blood and urine, were below the therapeutic  
228 range and in accordance with the literature [4-8]. Death was probably caused by the respiratory  
229 depression caused by propofol, assumed in uncontrolled conditions. The drug was probably  
230 assumed by an intravenous infusion. In fact the subject was a nurse and he was found in an  
231 operating room with a single sign of acupuncture in his arm. So suicidal hypothesis is the most  
232 likely.

233 Case 1 was more complicated. The very high concentration of propofol found in blood  
234 seemed incompatible with a single voluntary injection of propofol [23]. In fact propofol causes very  
235 rapid loss of consciousness. Even an intravenous infusion can hardly be responsible for a so high  
236 concentration.

237           Examining circumstantial data, the presence of several ampoules of “Propofol Kabi” in the  
238 room where the corpse was found, were evidenced. The corpse presented several signs of  
239 acupuncture. The police found out that the woman lived in the hotel room with her mother, also a  
240 nurse, in poor conditions; they gambled and had many debts. Probably they decided to both commit  
241 suicide, the mother injected some vials of propofol to the daughter but then changed her mind and  
242 did not kill herself. Death in the first case is then to be ascribed to an homicide rather than a suicide.  
243 In conclusion both deaths were related to propofol poisoning though with a different manner,  
244 homicide in Case 1 and suicide in Case 2. These considerations were deduced taking into account  
245 blood and urine concentrations of propofol. To confirm the poisoning caused by this drug, also the  
246 tissues available from the autopsy were analyzed. The presence of propofol was confirmed also in  
247 all the tissues considered.

248

## 249 **Conclusions**

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251           A liquid.liquid extraction protocol and a GC/MS and a Fast GC/MS-TOF method for the  
252 confirmation of propofol in the biological fluids was optimized and validated. The concentration of  
253 propofol was determined in blood, urine, bile, brain, liver and kidney of two suspected cases of  
254 poisoning caused by propofol. Data emerging from autopsy findings, histopathological exams and  
255 the concentrations of propofol evidenced by chemical and toxicological analysis, on the basis of  
256 literature data [4-16], allowed us to establish that both deaths were due to poisoning caused by  
257 propofol. In the first case the concentration of propofol in blood resulted to be 8.1 µg/mL while in  
258 the second one it was 1.2 µg/mL. The very different concentrations between the two cases were  
259 interpreted in two different ways: in the first case two females, mother and daughter, both nurses,  
260 decided to commit suicide with propofol, stolen by the daughter in the hospital where she worked.

261 The mother injected propofol in the ankle of the daughter, but then changed her mind and did not  
262 kill herself. In the second case a nurse committed suicide with an intravenous infusion of propofol.

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357 **Figure legends**

358 **Fig. 1** SCAN analysis of case 1 blood (a); Mass spectrum of propofol-TMS (b)

359 **Fig. 2** Chromatograms of blood of Case 1 in GC/MS (a) and GC/TOF (b) and urine in GC/MS (c)  
360 and GC/TOF (d). A=Propofol; B=capric acid; C=nicotine

361 **Fig. 3** Chromatogram for the determination of propofol in blood of Case 1.

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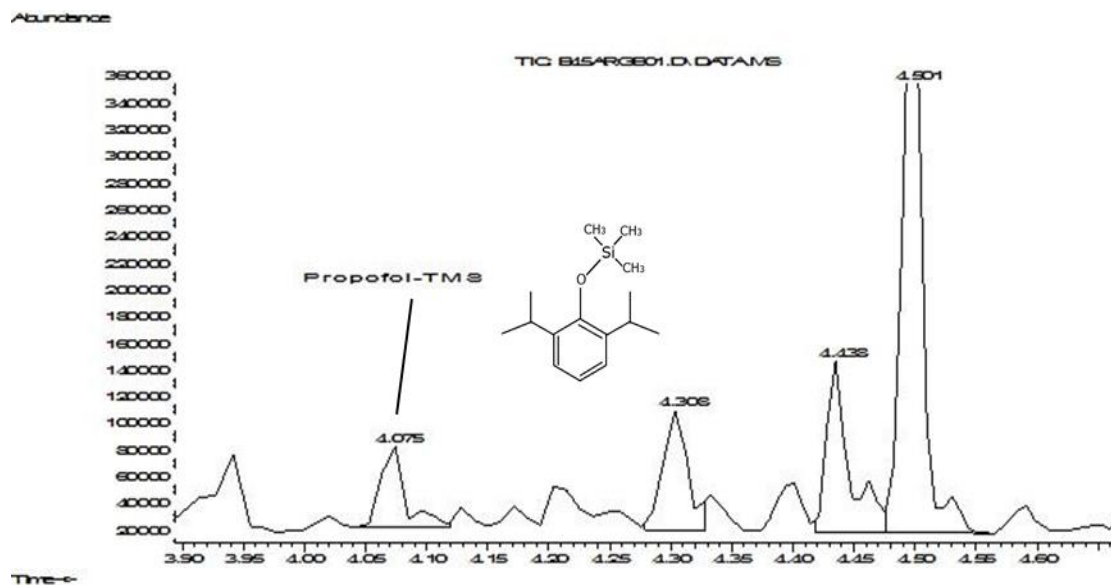
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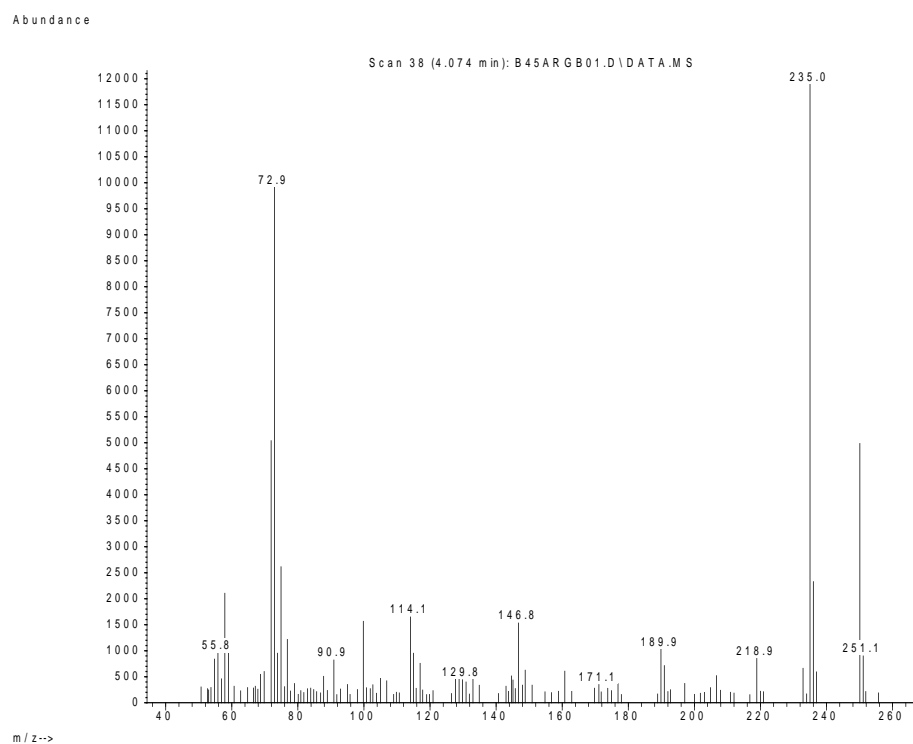
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371 a)



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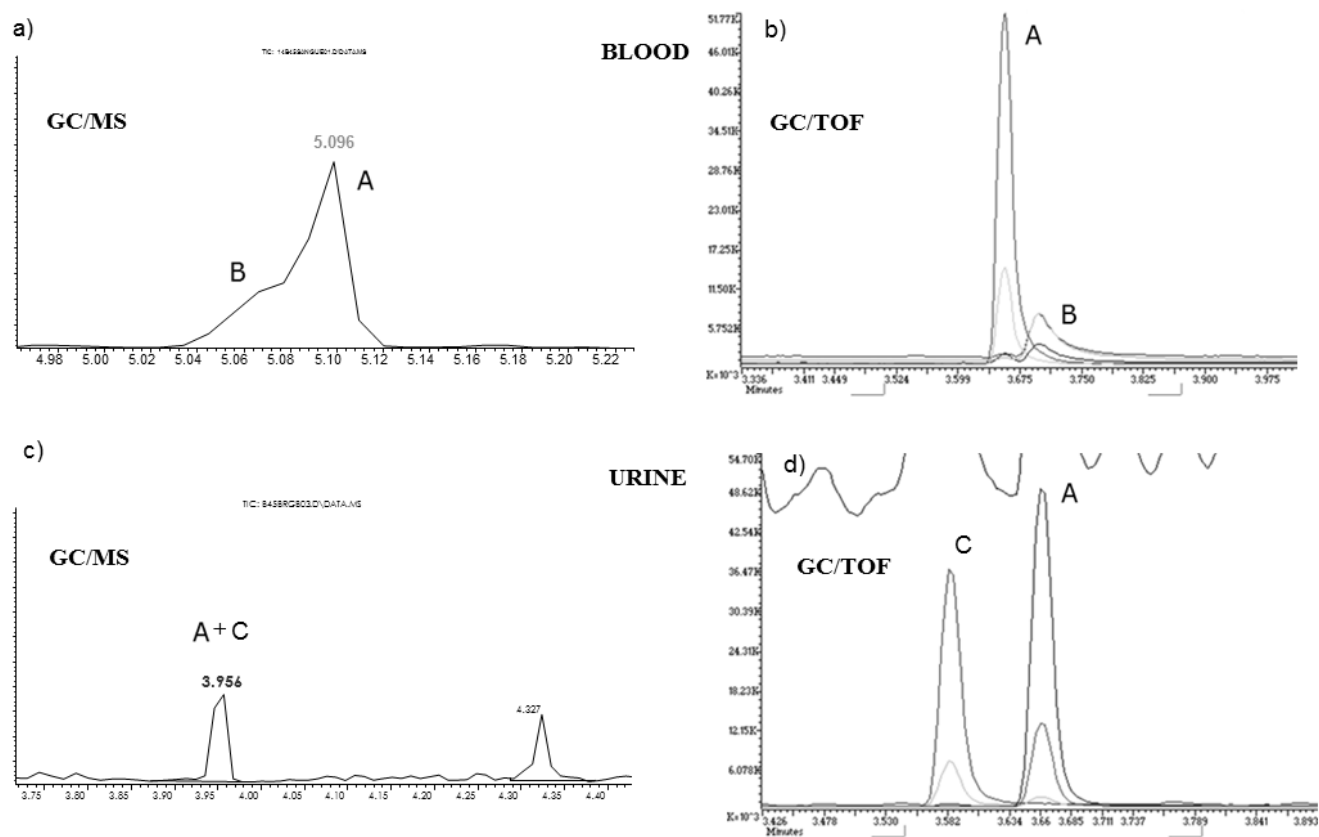
373 b)



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Fig. 1



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Fig. 2

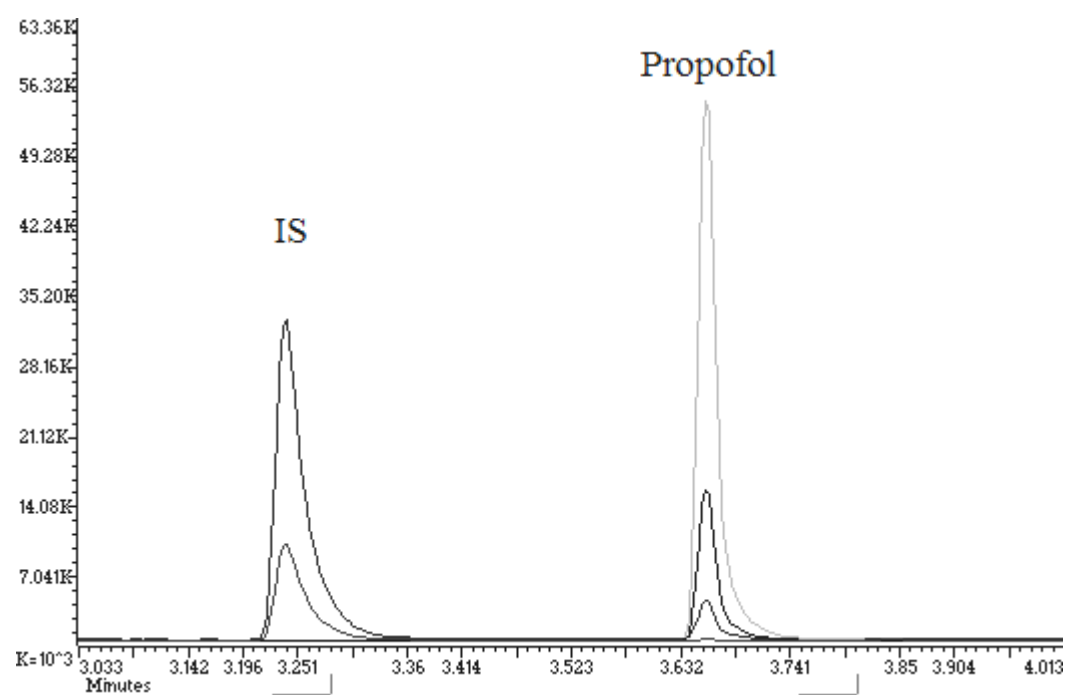


Fig. 3

404 **Table 1** Results of STA (n.d.= not determined)

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Specimen	Case 1	Case 2
Blood	Cotinine Caffeine	Cotinine Caffeine
Urine	Nicotine Cotinine Caffeine	Nicotine Caffeine
Bile	Nicotine Cotinine	n.d.

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410 **Table 2** Results of the quantitative determination of propofol in the biological specimens from the

411 two cases

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Specimen	Case 1 ( $\mu\text{g/mL}$ or $\mu\text{g/g}$ )	Case 2 ( $\mu\text{g/mL}$ or $\mu\text{g/g}$ )
Blood	8.1	1.2
Urine	0.21	0.0073
Hydrolyzed urine	1276.6	18.3
Bile	3.28	
Hydrolyzed bile	105.7	
Brain	31.1	4.7
Liver	52.2	49.1
Kidney		2.3

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